REMARKS

Applicants are amending their claims in order to further clarify the definition of various aspects of the present invention. Specifically, Applicants have amended each of claims 1 and 19 to recite that the individual chain molecule is immobilized on a (plastic) substrate surface, and to recite that the visualizing and identifying is performed by probing with a scanning probe microscope in solution so as to observe a profile of the plastic substrate surface having individual chain molecules immobilized thereon. Note, for example, page 17, lines 16-22, of Applicants' specification. Claims 2, 4, 21, 25, 27 and 28 have been amended in light of recitation of a substrate "surface" in claims 1 and 19.

In addition, Applicants are adding new claims 29-34 to the application. Claims 29 and 33, dependent respectively on claims 1 and 19, recite that the profile is observed using an atomic force acting between the plastic substrate surface having the individual chain molecules immobilized thereon and a probe of the scanning probe microscope; and claims 30 and 34, dependent respectively on claims 29 and 33, recite that the profile is observed by measuring an amount of flexing of the probe caused by the atomic force. Note, for example, page 17, lines 16-19, of Applicants' specification. Claims 31 and 32, each dependent on claim 1, further describe the substrate having chain molecules immobilized on the surface thereof, consistent with the description in the first paragraph on page 13, and in the last full paragraph on page 25, of Applicants' specification.

The concurrently filed RCE Transmittal is noted. It is respectfully submitted that the present amendments constitute the necessary Submission for this RCE Transmittal; and that in view of concurrent filing of this RCE Transmittal, entry of the

present amendments is clearly proper, notwithstanding the Finality of the Office Action mailed May 27, 2009.

Applicants respectfully submit that all of the claims presented for consideration by the Examiner patentably distinguish over the teachings of the documents applied by the Examiner in rejecting claims in the Office Action mailed May 27, 2009, that is, the teachings of the U.S. patent documents to Lee, et al., Patent No. 7,033,476, and to Obremski, et al., Patent Application Publication No. 2002/0001853, and the articles by Liu, et al., "Production of Nanostructures of DNA on Surfaces", in Nano Letters (2002), Vol. 2, No. 8, pp. 863-867, and Seong, et al., "Single-Molecular AFM Probing of Specific DNA Sequencing Using RecA-Promoted Homologous Pairing and Strand Exchange", in Analytical Chemistry, March 15, 2000, Vol. 72, No. 6, under the provisions of 35 USC 103.

It is respectfully submitted that the teachings of these documents as applied by the Examiner would have neither disclosed nor would have suggested such a molecular detection method as in the present claims, wherein the material visualized and identified is an individual chain molecule immobilized on a substrate surface and, as immobilized, is uprightly disposed relative to the substrate surface, with such visualizing and identifying of the individual chain molecule being performed by probing with a scanning probe electron microscope in solution so as to observe a profile of the surface having individual chain molecules immobilized thereon (see claims 1 and 19), especially, wherein the profile is observed using an atomic force acting between the plastic substrate surface having the individual chain molecules immobilized thereon and a probe of the scanning probe microscope (see claims 29 and 33), particularly wherein the profile is observed by measuring an amount of flexing of the probe caused by the atomic force (see claims 30 and 34).

In addition, it is respectfully submitted that these references as applied by the Examiner would have neither disclosed nor would have suggested such molecular detection method as in the present claims, having features as discussed previously in connection with claims 1 and 19, and, moreover, wherein the chain molecule immobilized on the substrate, which is visualized and identified, is a nucleic acid (see claim 19), and/or wherein the substrate is a plastic substrate (see claim 1; note also claim 26).

Additionally, it is respectfully submitted that the teachings of the applied documents would have neither disclosed nor would have suggested such molecular counting method as in the present claims, including detecting a molecule by the method of claims 1 and 19, and counting the number of detected chain molecules per unit area (see claims 6, 7, 23 and 24); or the molecular localization detection method as in the present claims, wherein counting of the number of detected chain molecules per unit area gives molecular localization information (see claims 7 and 24).

Furthermore, it is respectfully submitted that the teachings of these applied documents would have neither disclosed nor would have suggested such a production process for a substrate with a chain molecule immobilized thereon, this process including the method according to claim 1 or 19 (see claims 17 and 25).

In addition, it is respectfully submitted that the teachings of the applied documents would have neither disclosed nor would have suggested such molecular detection method as in the present claims, having features as discussed previously in connection with claims 1 and 19, and, additionally, wherein the chain molecule is an uprightly disposed single strand molecule (note claim 2), especially wherein the uprightly disposed single strand molecule is a molecule selected from the specific

group of substances as in claim 3; and/or wherein the chain molecule immobilized on the substrate surface is a multiple strand molecule comprising an uprightly disposed single strand molecule (comprising nucleic acid) and at least one chain molecule that can bind to the single strand molecule (can bind to the nucleic acid), as in claims 4 and 21; and/or wherein the multiple strand molecule is a complex of at least one molecule selected from a specific group thereof as in claims 5 and 22; and/or wherein the individual chain molecule, as immobilized, is uprightly disposed relative to the substrate surface so as to extend substantially perpendicularly from the substrate surface (see claims 27 and 28); and/or the more specific definition of the substrate having chain molecules immobilized on the surface thereof, as in claims 31 and 32.

The present invention relates to a molecular detection method, which can be used to visualize and identify localized chain molecules, and a molecular counting method and molecular localization detection method using such molecular detection method.

In immobilizing, e.g., DNA on a substrate, there are known techniques in which DNA is directly synthesized on a substrate, and in which DNA, that has been synthesized separately, is immobilized on a substrate. In each of these techniques, unless the DNA is uniformly and distributedly (nonlocalized) immobilized on an intended section on the substrate, qualitative and quantitative analytic performance cannot be exhibited. Conventionally, there is no technique for examining, at the molecular level, whether or not, e.g., single strand DNA is uniformly immobilized on a specific area on the substrate, and there has been a desire for development of such a testing technique.

As described in the paragraph bridging pages 2 and 3 of Applicants' specification, with regard to means for obtaining information on whether or not immobilized molecules are nonlocalized or localized on a substrate, there is an observation using an electron microscope; however, since this observation is carried out under vacuum, in the case of biopolymers the structure thereof will be destroyed and observation is not possible, or they stick to the substrate, thus making it impossible to distinguish them from the substrate.

Thus, there is a desire for a molecular detection technique that enables, in a substrate such as a DNA chip or a DNA microarray in which a large number of chain molecules are immobilized, the individual molecules to be visualized and counted while maintaining activity of the chain molecules; and, moreover, wherein information about localization of the molecules can be obtained.

Against this background, Applicants provide a technique achieving the objects referred to previously. Specifically, Applicants provide a technique wherein an individual chain molecule is visualized and identified easily and accurately, with such individual chain molecule immobilized on a surface of, e.g., a plastic substrate and being immobilized uprightly disposed relative to the substrate surface, the visualizing and identifying being performed by probing with a scanning probe microscope in solution, so as to observe a profile of the substrate surface having the individual chain molecules immobilized thereon.

Accordingly, by the present invention, especially when using a plastic substrate, a relatively inexpensive substrate is utilized; and, moreover, the chain molecules immobilized on the substrate surface, and uprightly disposed relative thereto while immobilized, can be detected easily and accurately, using a scanning probe microscope and observing a profile of the substrate surface having the

individual chain molecules immobilized thereon, and the molecules can be identified, with information about localization obtained.

Moreover, since the molecules can be individually recognized, as compared with conventional detection methods wherein an array is visualized, detection is possible with a small amount of sample, and a high sensitivity detection method is achieved. Note, for example, the paragraph bridging pages 25 and 26 of Applicants' specification.

Furthermore, since the scanning probe microscope is used so as to observe a profile of the substrate surface having individual chain molecules immobilized thereon, the present method can be utilized to visualize, e.g., DNA-protein complexes formed by the pairing of RecA-coated complementary short single-stranded DNA probes with linear double-stranded DNA targets, and can identify the sequence-specific site complementary to the single-stranded DNA probe on the double-stranded DNA target. In this regard, and as will be discussed further *infra*, in Seong, et al. a profile of the side of the detection target is utilized. If the liner double-stranded DNA of Seong, et al. were uprightly disposed relative to the substrate, the atomic force microscope could not observe the profile of the side of the liner double-stranded DNA; and, as a result, Seong, et al. could not visualize the DNA-protein complexes formed by the pairing of RecA-coated complementary short single-stranded DNA probes with linear double-stranded DNA targets, and could not identify the sequence-specific site complementary to the single-stranded DNA probe on the double-stranded DNA target.

Liu, et al. discloses three atomic force microscopy (AFM)-based lithography techniques for creating nanopatterns of self-assembled monolayers (SAMs) and biosensors: nanoshaving, nanografting and nanopen reader and writer (NPRW).

This article goes on to disclose that using these methods, nanostructures of thiols as small as 2 x 4 nm^2 have been successfully produced with various chain lengths and terminal groups; that high-resolution images show that the thiol molecules within the nanopatterns are closely packed; and that in addition to SAM structures, biomolecules such as proteins can be positioned on a surface via selective immobilization. Note the paragraph bridging the left- and right-hand columns on page 863, as well as the sole full paragraph in the right-hand column on page 863. Note also the last full paragraph in the left-hand column on page 864, as well as Fig. 2 on page 864. The Abstract of Liu, et al. discloses that nanopatterns of thiolated single-stranded DNA (ssDNA) are produced by using the AFM-based lithography technique; the ssDNA molecules adsorb chemically onto an exposed gold area through a sulfur headgroup, and the ssDNA molecules stand up on the gold surfaces and adapt a stretched conformation.

Liu, et al. is concerned with a nanopattern forming technique, attempting to produce the nanopatterns of single-stranded DNA. When producing the nanopatterns of the single-stranded DNA molecules, one of ordinary skill in the art need not visualize an individual single-stranded DNA molecule. Furthermore, if one of ordinary skill in the art visualized the individual single-stranded DNA molecule within the nanopattern, then one could not observe the entire structure of the nanopattern (e.g., square, rectangular or linear). Thus, it is respectfully submitted that this reference would have neither disclosed nor would have suggested such a <u>molecular detection method</u> as in the present claims, including the visualizing and identifying of an <u>individual</u> chain molecule immobilized on a (plastic) substrate surface and (while immobilized) uprightly disposed relative to the substrate surface, with the visualizing and identifying being performed by probing with a scanning probe

microscope in solution, so as to make the observation of the profile of the substrate surface having the individual chain molecules thereon, as in the present claims, or other features of the present invention as discussed previously, and advantages thereof. In this regard, it is emphasized that Liu, et al. discloses visualizing and identifying the aggregates of the thiolated ssDNA molecules produced by using the alkanethiol SAMs.

It is respectfully submitted that the secondary references applied by the Examiner together with Liu, et al. would not have rectified the deficiencies thereof, such that the presently claimed invention as a whole would have been obvious to one of ordinary skill in the art.

Obremski, et al. discloses ligand binding assays in which an analyte is detected and quantified, directly or indirectly, on the basis of its specific affinity for a chemically modified solid material. Note paragraph [0002] on page 1 of this patent document. In the method described in this patent document, an array of sorbent zones is immobilized on a substrate, the sorbent zones including an analyte binding partner, which can be an oligonucleotide probe, antibody, or receptor molecule. When a defined volume of sample, believed to contain an analyte, is deposited on a sorbent zone, the analyte is substantially depleted from the sample to form an analyte capture complex with the analyte binding partner. In one embodiment disclosed in this patent document, the sorbent zones also contain a first binding partner attached to the substrate, wherein the first binding partner forms a first binding complex with a conjugate, the conjugate comprising a first ligand and the analyte binding partner, the first ligand binding specifically with the first binding partner and the analyte binding partner can bind specifically with the analyte. Note paragraphs [0009]-[0011] on page 1 of this patent document. See also

paragraph [0015] on page 2 of this patent document, disclosing preferred substrates selected from the group consisting of polycarbonate, polystyrene, polyethylene, polypropylene and polymethylmethacrylate.

Even assuming, arguendo, that the teachings of Obremski, et al. were properly combinable with the teachings of Liu, et al., such combined teachings would have neither disclosed nor would have suggested the presently claimed invention, including visualizing and identifying an individual chain molecule, immobilized on the substrate, and, while immobilized, being uprightly disposed relative to the substrate, by probing with a scanning probe microscope in solution so as to observe a profile of the surface, of the substrate, having the individual chain molecules immobilized thereon; or the other features of the present invention as discussed previously, and advantages thereof.

Seong, et al. discloses an attempt to visualize, by imaging in solution using atomic force microscopy (AFM), DNA-protein complexes formed by the pairing of RecA-coded complementary short single-stranded DNA probes with linear double-stranded DNA targets, and to identify the sequence-specific site complementary to the single-stranded DNA probe on the double-stranded DNA target. Note the first full paragraph in the left-hand column on page 1298. In the Abstract of this article by Seong, et al., it is disclosed that the specific sequence in a linearlized double-stranded DNA target has been identified at a single-molecular level by AFM, accomplished using RecA-coded, single-stranded DNA probes which were paired with a specific complementary DNA sequence in a linear double-stranded DNA target by strand-exchange reaction at a homologous sequence site with target DNA.

Thus, the Seong, et al. article attempts to visualize the DNA-protein complexes formed by pairing of RecA-coded complementary short single-stranded

DNA probes with linear double-stranded DNA targets and to identify the sequence-specific site complementary to the single-stranded DNA probe on the double-stranded DNA target. It is respectfully submitted that the Seong, et al. teaches visualizing and identifying the DNA-protein complexes absorbed parallel to the mica surface in order to identify the sequence-specific site on the double-stranded DNA target. Note, in particular, Figs. 4, 6 and 7 of Seong, et al., on pages 1291 and 1292 thereof. It is respectfully submitted that if Seong, et al. were combined with Liu, et al., as applied by the Examiner, the sequence-specific site on the double-stranded DNA target would not be identified. Thus, it is respectfully submitted that one of ordinary skill in the art would not have combined the teachings of Seong, et al. and of Liu, et al., as applied by the Examiner, as this would destroy Seong, et al. for its intended purpose. See In re Ratti, 123 USPQ 349 (CCPA 1959).

In addition, it is emphasized that in Seong, et al., the visualizing and identifying is of DNA-protein complexes adsorbed parallel to the mica surface. It is respectfully submitted that the combined teachings of the references as applied by the Examiner in rejecting claims in Item 9 on pages 3-8 of the Office Action mailed May 27, 2009, would have neither disclosed nor would have suggested such visualizing and identifying of immobilized individual chain molecules uprightly disposed relative to the substrate, and advantages achieved due thereto.

With respect to the rejection of claims in Item 10 on pages 8 and 9 of the Office Action mailed May 27, 2009, Obremski, et al. has been previously discussed.

Lee, et al. discloses separation, detection and counting of single molecules at nanometer scale, in particular wherein molecular separation is achieved by driving single molecules through a microfluidic or nanofluidic medium using programmable and coordinated electric fields, and detecting the molecules using nanoelectrode-

gated tunneling methods, dielectric monitoring and other methods. The technique disclosed in Lee, et al. is described in the paragraph bridging columns 3 and 4 of this patent, and includes, inter alia, a pair of nanoelectrodes forming a nanoscale detection gate therebetween; a programmable pulse generator connected to produce an electrophoresis electric field between macroelectrodes, the electrophoresis electric field being capable of controllably moving molecules in the liquid along means for accommodating a liquid through the detection gate; and a second programmable pulse generator connected to produce a holding electric field between the electrically conductive plates, the holding electric field capable of holding and/or orienting molecules in the liquid with respect to the base, with a molecule detection means connected to the nanoelectrodes. Note also, in a disclosure of a first embodiment of the invention in this patent, and with reference to Figs. 4-7 thereof, this patent discloses that the separation and counting of single molecules is achieved by electrophoresizing sample molecules 47 through a thin liquid layer 48, also called a micro/nanofluidic separation column. The number of sample molecules 47 can be counted one by one as each of them passes through the detection gate 42. Note column 5, lines 40-50.

It is emphasized that according to Lee, et al., the sample molecules are counted one by one as each of them passes through the detection gate. That is, in Lee, et al. the single molecules have to move on the surface of the substrate. It is respectfully submitted that the teachings of this patent would have neither disclosed nor would have suggested, and in fact would have taught away from, such method as in the present claims, including, inter alia, wherein an individual chain molecule immobilized on a substrate surface, and uprightly disposed relative to the substrate surface while immobilized, is visualized and identified by probing with a scanning

probe microscope in solution, especially with the observation of the profile as in the present claims, or other features of the present invention as discussed previously.

Emphasizing in particular that in Lee, et al. the molecules pass one by one for detection, one of ordinary skill in the art would not have looked to the teachings of Obremski, et al., applied by the Examiner in combination with the teachings of Lee, et al.; and, in any event, even assuming, arguendo, that the teachings of Lee, et al. and of Obremski, et al. were properly combinable, the combined teachings would have neither disclosed nor would have suggested the presently claimed molecular detection method, including, inter alia, visualizing and identifying individual chain molecules immobilized on a substrate surface and uprightly disposed relative to the substrate surface while immobilized, by probing with a scanning probe microscope in solution so as to make the observation of the profile of the surface having the chain molecule immobilized thereon, as in the present claims, and other features of the present invention as discussed previously, and advantages due thereto.

The contention by the Examiner in the second paragraph on page 10 of the Office Action mailed May 27, 2009, that Liu, et al. teaches a single-stranded DNA molecule immobilized on a substrate uprightly disposed relative to the substrate and visualizing and identifying these molecules by scanning probe microscope in solution, the Examiner referring to Figs. 1 and 3A of Liu, et al., is respectfully traversed. As mentioned previously, Liu, et al. attempts to produce nanopatterns of single-stranded DNA; and when producing such nanopatterns, one of ordinary skill in the art need not visualize an individual single-stranded DNA molecule. Furthermore, if one sought to visualize an individual single-stranded DNA molecule within the nanopattern, then one could not observe the entire structure of the nanopattern. Thus, contrary to the contention by the Examiner, Liu, et al. would have neither

taught nor would have suggested the visualizing and identifying as in the present invention.

The further contention by the Examiner in the paragraph bridging pages 10 and 11 of the Office Action mailed May 27, 2009, that Seong, et al. is relied upon for individual molecule analysis, is noted. However, it must be emphasized that according to Seong, et al., the DNA-protein complexes are absorbed parallel to the mica surface, with observation of the side. If the liner double-stranded DNA of Seong, et al. were uprightly disposed relative to the substrate, the atomic force microscope could not observe the profile of the side of the liner double-stranded DNA; and, as a result, Seong, et al. could not visualize the DNA-protein complexes.

It is respectfully submitted that one of ordinary skill in the art concerned with in Liu, et al., would not have looked to the teachings of Seong, et al. However, if the teachings of these references were combined, such combined teachings would have neither disclosed nor would have suggested an individual chain molecule immobilized on a substrate surface and as immobilized being uprightly disposed relative to the substrate surface, with the visualizing and identifying being performed by probing with a scanning probe microscope in solution so as to observe a profile of the substrate surface having individual chain molecules immobilized thereon.

It is respectfully submitted that the Examiner is using bits and pieces of the teachings of the applied references, using Applicants' disclosure as a map, contrary to the requirements of 35 USC 103.

In the paragraph bridging pages 11 and 12, as well as in the first full paragraph on page 12, of the Office Action mailed May 27, 2009, the Examiner appears to require Applicants to provide evidence as to why the teachings of the secondary references in the method of the primary references "won't work", in order

to rebut the Examiner's conclusion of obviousness. It is respectfully submitted that the Examiner is applying an improper standard under the requirements of 35 USC 103. That is, it is respectfully submitted that the Examiner must provide some reason for combining teachings of the applied references, which reason must be independent of they disclosure of Applicants' invention in their application. It is respectfully submitted that applying the proper standard under 35 USC 103, the Examiner has not established obviousness of the presently claimed subject matter.

In view of the foregoing comments and amendments, and in light of the concurrently filed RCE Transmittal, entry of the present amendments, and reconsideration and allowance of all claims being considered on the merits in the above-identified application, are respectfully requested.

To the extent necessary, Applicants hereby petition for an extension of time under 37 CFR 1.136. Kindly charge any shortage of fees due in connection with the filing of this paper, including any extension of time fees, to the Deposit Account of Antonelli, Terry, Stout & Kraus, LLP, Account No. 01-2135 (case 1204.45527X00), and please credit any overpayments to such Deposit Account.

Respectfully submitted,

ANTONELLI, TERRY, STOUT & KRAUS, LLP

By **/William I. Solomon/**
William I. Solomon
Registration No. 28,565

WIS/ksh
1300 N. 17th Street, Suite 1800
Arlington, Virginia 22209
Tel: 703-312-6600
Fax: 703-312-6666